THE TOXICITY TOWARDS STAPHYLOCOCCUS OF DILUTE PHENOL SOLUTIONS CONTAINING SODIUM BENZOATE

BY KENNETH E. BURGESS

The irregularities observed by Lemon[1] in his experiments on *staphylococcus* with low concentrations of phenol were referred by Laird[2] to plasmolysis of the cocci due to the low osmotic pressure of the solutions employed; if this explanation be the right one, addition of any indifferent substance in suitable concentration ought to remove the abnormality. At the suggestion of Prof. Lash Miller I put this conclusion to the test, in the winter of 1913–4.

Sodium benzoate was chosen, as that salt had less effect on the toxicity of phenol towards anthrax than any other of the dozen salts studied by Paul and Krönig; and as, according to Laird, two atmospheres is the lowest osmotic pressure that is at all safe, a twentieth-normal solution was employed. Blank experiments showed that decinormal sodium benzoate was better for the cocci; but unless a number of equilibrium determinations were to be made, it was obviously better to keep down the concentration of the salt.

Time	0	20	35	40	45	50	55	60	Minutes
N/30 sod. ben.	3000	1500	1500	—	100	—	55	—	colonies
N/20 sod. ben.	3000	2500	—	2500	—	1500	—	1000	colonies
N/10 sod. ben.	2000	1500	1500	—	—	1500	—	900	colonies
N/12 NaCl	3000	3000	3000	—	2500	2500	—	—	colonies

The determinations were made with a 24-hour culture of *staphylococcus pyogenes aureus*, following the technique used by Lemon; but much time and labor was saved by adding methylene-blue to a drop of the liquid and examining it under the microscope before pouring a plate. A high magnifying power was needed for these small objects (oil immersion,

---

[1] Jour. Phys. Chem., **24**, 570 (1920).

[2] Ibid., **24**, 664 (1920).

1000 to 2000 diam.) and there were usually only about a dozen cells in the field, so no attempt was made to institute a quantitative comparison of the results obtained by the two methods; as my object in using the dye was merely to get a hint when to stop, no record has been kept of these observations. The results of one of the preliminary trials of the method, however, have been preserved; in this experiment the number of colonies on the plates was larger than is convenient, and evidently no great pains were taken in counting them, but it may serve as an illustration of the use of the stain in connection with plating work. Two fields were examined under the microscope; the poison was 0.4% phenol.

Time	0	10	20	30	40	40	50	60	70	80	90	Minutes
In field (1)	—	8	8	16	14	16	20	10	20	8	8	cells
of wh. stained	—	0	1	4	3	2	5	5	13	7	8	cells
In field (2)	—	10	10	20	15	18	22	12	25	9	9	cells
of wh. stained	—	1	0	5	4	3	6	6	14	8	9	cells
On plate	—	500	500	400	500	300	500	500	100	60	0	colonies

The results in each of the following tables were obtained with cocci from the same suspension; two plates were poured at each interval, the readings are separated by a comma. In each table the least toxic solution is put first: "Ph 0.1" means 0.1 g phenol per 100 cc; "B" that the solution was twentieth-normal with respect to sodium benzoate.

An experiment in which 1.25% phenol was used, with and without sodium benzoate, gave thickly sown plates immediately after poison and yeast had been brought together, but three minutes later all the cocci were dead.

Time	0	15	25	Minutes
Ph 1.0 B	600, 600	10, 12	0, 0	colonies
Ph 1.0	600, 650	5, 6	0, 0	colonies

Time	0	15	30	45	60	75	Minutes
Ph 0.75 B	250, 240	275, 200	90, 80	40, 45	1, 0	0, 0	colonies
Ph 0.75	300, 310	130, 135	20, 18	2, 1	0, 0	—	colonies

Time	0	15	25	35	45	55	60	Minutes
Water	50, 55	30,35	25,25	25, 28	8, 6	6, 7	0, 0	colonies
Ph 0.25; B	135, 130	50,40	40, 40	30, 25	10, 9	0, 0	—	colonies
Ph 0.25	80, 90	25, 25	6, 7	1, 0	0, 0	—	—	colonies

Time	0	15	30	40	50	60	Minutes
Ph 0; B	210, 200	195, 190	190, 190	165, 155	160, 160	150, 140	colonies
Ph 0.20; B	210, 200	120, 125	125, 120	120, 115	115, 115	115, 110	colonies
Water	220, 200	155, 150	145, 140	95, 85	45, 50	35, 40	colonies
Ph 0.20	200, 215	175, 165	—	75, 70	60, 60	6, 5	colonies

As was to be expected from the results with anthrax, and from Laird's distribution experiments, this low concentration of sodium benzoate has hardly any effect on the toxicity of 1.0% phenol; its increasing efficiency as a life preserver in the more dilute solutions is in full accord with the explanation put forward by Laird.

In the final set, the toxicity of 0.1, 0.3 and 0.5% phenol in presence of $N/20$ sodium benzoate was compared; all abnormality has disappeared, 0.1% phenol has practically no effect and increase in the concentration of the phenol hastens death.

Time	0	15	20	45	55	Minutes
Ph 0; B	350, 400	—	100, 125	5, 3	0, 0	colonies
Ph 0.10; B	750, 800	—	350, 400	5, 6	0, 0	colonies
Ph 0.20; B	250, 300	—	50, 60	0, 0	—	colonies
Ph 0.50; B	350, 400	0, 0	—	—	—	colonies

Summary

Determinations of the rate at which staphylococcus is poisoned by dilute solutions of phenol with and without addition of sodium benzoate, confirm the conclusion that the abnormalities observed by Lemon were due to plasmolysis.

Addition of methylene-blue and examination under the microscope before pouring the plates saves much time and prevents disappointment in work of this kind.

The University of Toronto
July 1920